IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| In re Application of: | ) | Atty. Docket No.: **STURK0003** |
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| Ralf RESKI et al. | ) | Confirmation No. 9421 |
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| Serial No. 10/089,450 | ) | Group Art Unit: 1638 |
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| Filed: March 29, 2002 | ) | Examiner: KUBELIK, Anne R. |
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| For: METHOD FOR THE PRODUCTION OF PROTEINACEOUS SUBSTANCES | ) | |

**DECLARATION UNDER 37 C.F.R. § 1.132**

**MAIL STOP:**

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1. I, Klaus von Schwartzenberg, state that I am an expert in the field of plant cell cultivation/development research and transformation. A copy of my Curriculum Vitae is attached herewith as evidence of my relevant expertise.

2. I have read and I am familiar with the article by N. Houba-Hérin, "*Cytokinin oxidase from Zea mays: purification, cDNA cloning and expression in moss protoplasts*," 17(6) THE PLANT JOURNAL 615 (1999)(of record, hereafter the "Houba-Hérin article"). Additionally, I have been working in the field of cytokinin metabolism since 1991. From 1993 to 1995 I worked as a postdoctoral scientist with the research group at the Laboratoire de Biologie Cellulaire INRA, which published the Houba-Hérin article. Although I did not directly participate in the experiments that were published as the Houba-Hérin article, I am aware of the purpose of the experiments based on my experience in the field of cytokinin research, and I am aware of what the Houba-Hérin article would teach to one of ordinary skill in this technology.

3. In this declaration, I review the Houba-Hérin article, and based on my knowledge and belief, provide my testimony regarding the experiments published in the Houba-Hérin article, as well as the scope and limitations of the teachings of the Houba-Hérin article. Results concerning the field in question, which were published later than the Houba-Hérin article, are not taken into account for this declaration.

4. I believe, based on my reading of the Houba-Hérin article, that it relates to the purification and cloning of cytokinin oxidase (CKO) from Zea mays. The functional characterization was subsequently investigated in moss protoplasts (See Abstract). I believe that the Houba-Hérin article teaches a metabolic assay based on transient expression of the CKO enzyme in moss protoplasts thereby demonstrating the functionality of the recombinant enzyme (See Abstract). Moss protoplasts were chosen, in my opinion, because they represent the most simple plant organisms enabling correct plant-specific processing which was held to be necessary to achieve CKO functionality. The Houba-Hérin article teaches generation of moss protoplasts according to the method of Schaefer and Zyrd (1997) and to effect transformation of protoplasts according to Schaefer et al. (1994)( Houba-Hérin article, at 624, col. 2, lines 42-45; and at 625, col. 2, lines 36-37). The Houba-Hérin article also teaches to centrifuge the transformed protoplasts in order to obtain samples from the supernatant after a cultivating period of 44 hours (Houba-Hérin article, at 624, col. 2, lines 46-48). The successful expression and the functionality of the recombinant enzyme were demonstrated using an assay in which the conversion of the radioactive labeled substrate isopentenyladenosine was measured (Houba-Hérin article, at 622, col. 2, lines 46-54). The assay employed only $1/40^{th}$ of the substrate as compared to the standard assay (See Table 1). In my opinion, these facts indicate that the authors of

the Houba-Hérin article expected to achieve a detectable, but low expression efficiency.

5. On the other hand, it is my opinion, based on my reading of the Houba-Hérin article and based on my experience in the field of cytokinin metabolism, that the Houba-Hérin article is directed to the demonstration of the functionality of the cloned CKO gene, but it is not directed to a method for the expression and secretion of recombinant proteins in liquid culture medium. Evidence for my opinion can be seen in the fact that the article does not even mention the provision of a signal or leader peptide. Although the authors mainly recovered CKO activity in the culture medium, they did not investigate whether the enzyme was liberated in the medium by cell lysis or whether it was excreted (Houba-Hérin article, at 621, col. 2, lines 10-12, "Either the enzyme is liberated in the medium by cell lysis or it is excreted."). Since the culture medium containing the transformed protoplasts was collected after centrifugation, it seems to a great extend possible, in my opinion, that the enzyme was liberated into the medium by cell lysis caused by shear forces during centrifugation. Although this could have been investigated by assaying the supernatant for GUS activity, these experiments were not conducted, thereby providing further evidence

from which a person of ordinary skill in the art would reasonably infer that the authors did not provide a reliable method for expressing and secreting recombinant proteins in the culture medium of transformed moss protoplasts. In other words, I believe that the investigators, at the time they performed their experiments, only sought to achieve expression of a glycosylated protein, irrespective of whether its activity was found in the cells or elsewhere. In addition, the authors refer to the results from Kaminek and Armstrong, obtained from analysis of glycosylated and unglycosylated CKOs in *Phaseolus* callus tissues, suggesting "that a compartmentation could exist in the cells that keep the glycosylated form in the cell wall or plasmalemma and the unglycosylated form in an internal compartment" (Houba-Hérin article, at 621, col. 2, last para.). Accordingly, it is my opinion, based on the expressed teachings of the Houba-Herin article, that it would be entirely unsupported speculation to say that the CKO activity definitely represented extracellular protein and not protein released from lysed cells.

6. It is my belief, from my reading of the Houba-Herin article, that from the viewpoint of one of ordinary skill in the art the goal of the work group was to ensure the correct, i.e. plant-specific, folding and glycosylation of CKO. In order to achieve this goal, the authors used a transient expression assay in *P. patens* protoplasts (See

Houba-Hérin article, at 619, col. 2, lines 24-27). In other words, in my opinion the investigation by Houba-Hérin and co-workers should not be interpreted, and would not be interpreted by a person of ordinary skill in the art, as a protocol for the development of a process or a construct in which recombinant proteins are secreted into culture medium.

**Summary**

7. It is my opinion, based on the Houba-Hérin article and other evidence, such as my personal experience in the field of plant cell culture, that:

a. the scope of the experiments provided by the Houba-Hérin article is limited to demonstrating the transient expression of CKO enzyme in moss protoplasts in order to demonstrate the functionality of the recombinant enzyme;

b. the Houba-Hérin article does not demonstrate that the CKO enzyme activity in culture media is the result of cell lysis or excretion; and

c. in accordance with the authors' opinion (Houba-Hérin article, at 621, col. 2, lines 11-12, "Either the enzyme is liberated in the medium by cell lysis or it is excreted.") with which I concur, the probability was considerably high that liberation of CKO enzyme into the culture medium was the result of cell lysis upon the step of centrifugation.

8. I declare under penalty of perjury that the foregoing is true and correct, that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed by,

Date: 05.09.2006 Name: K. v. Schwartzenberg

KLAUS VON SCHWARTZENBERG

## Dr. rer. nat. Klaus von Schwartzenberg

## Curriculum Vitae

**Born in Aachen, Germany, on 7th March 1959, married, three children.**

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| **Today** | **17** publications, thereof **15** related to cytokinin physiology/metabolism in plants, **7** related to research on bryophytes |
| | since 9/97 permanent position as scientist/teacher at Biocenter Klein Flottbek and Botanical Garden, University of Hamburg |
| | teaching activities about plant science and molecular cell biology |
| | research area: Cytokinins and secondary metabolism in the moss *Physcomitrella patens* |
| 12/95 - 08/97 | scientist at Institute for Plant Physiology and Mikrobiology, Freie Universität Berlin |
| 12/1993 - 11/95 | postdoctoral research stay at "Institut National de la Recherche Agronomique" (INRA) Versailles (France), Laboratoire de Biologie Cellulaire, working area „Metabolism of cytokinins in the moss *Physcomitrella patens*". |
| 5/1991 - 11/1993 | postdoctoral research stay at INRA Orléans, working area "Metabolism of cytokinins in Norway Spruce and *IPT*-transgenic Poplar" |
| 9/1989 - 10/1990 | scientist at University of Hamburg, Institute of Applied Botany, research area: tree physiology |
| 1989 | Ph.D Degree (Dr. rer. nat.) at University of Bonn, Thesis on enzyme immunological measurements of plant hormones in forest trees. |
| 7/1984 - 11/1985 | Civil service, department of pneumology and allergology of University children´s hospital, Bonn |
| 1978 – 1984 | Studies in Biology, at Universities of Aachen and Göttingen |

## publication list


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4. Klämbt D., H. Hahn, K. v. Schwartzenberg: Zur Hormonphysiologie umweltgeschädigter Fichten. in: Rheinische Friedrich-Wilhelms-Universität Bonn (Hrsg.): Verantwortung für die Zukunft - Klima und Umweltforschung an der Universität Bonn, p 128-129, Bonn (1992).

5. Schwartzenberg v. K., H. Hahn: The cytokinin content in needles of Norway spruce (*Picea abies* (L.) Karst.) with different degrees of damage. Journal of Plant Physiology 139, 218-223 (1991).

6. Schwartzenberg v. K., P. Doumas, L. Jouanin, G. Pilate: Enhancement of the endogenous cytokinin content in poplar by transformation with the T-DNA gene ipt. Tree Physiology 14, 27-35 (1994).

7. Schwartzenberg v. K., M. Bonnet-Masimbert, P. Doumas: The isolation of two cytokinin metabolites from the rhizosphere of Norway spruce seedlings (*Picea abies* L. Karst.). Plant Growth Regulation 15, 117-124 (1994).

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10. Schulz P, R Reski, R. Maldiney, M. Laloue, K. v Schwartzenberg: Kinetics of cyokinin production and bud formation in Physcomitrella : analysis of wild type, a developmental mutant and two of its ipt transgenics. J Plant Physiol 156, 768-774 (2000).

11. Moffatt B.A., L. Wang, M.S. Allen, YY. Stevens, W. Qin, J . Snider, K. v. Schwartzenberg: Adenosine kinase of Arabidopsis. Kinetic properties and gene expression. Plant Physiol. 124, 1775-1785 (2000).

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13. Schwartzenberg v. K., C. Pethe, M. Laloue: Cytokinin metabolism in *Physcomitrella patens* – differences and similarities to higher plants. Plant Growth Regulation 39, 99-106 (2003).

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